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FRUIT SETTING

of the

DELICIOUS APPLE

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As Affected by Cytological Changes Within the Flowers and Young Fruits

FRED O. HARTMAN AND FREEMAN S. HOWLETT

INTRODUCTION

During the past half century fruit growers and pomologists have from time to time devoted much attention to the problem of fruit-set of the commercial apple varieties. In many instances it was found that the unsatisfactory fruit-set of these varieties had resulted from a lack of varieties suitable for cross-pollination, varieties not having blooming periods which overlapped sufficiently, the presence of an inadequate supply of pollinizing insects, and various other factors. In addition to these factors which bring about an unsatisfactory setting of fruit, weather conditions during the blooming season, such as temperature, rainfall and humidity also played an important role in governing the extent of fruit-set.

Supplementing the above environmental conditions which may cause an unsatisfactory fruit-set, there is, moreover, the presence of inherent factors such as are found in Stayman Winesap, Rhode Island Greening and other triploid varieties; namely, the production of non-functional pollen and egg cells and irregularities in the development of the embryo sacs.

In recent years, the variety Delicious, a diploid, has created considerable concern among orchardists and pomologists in regard to its unreliable fruit-setting characteristic. Delicious has been observed to be less resistant to low temperatures just before and during bloom than most other commercial apple varieties. Roberts (35) reported that spring frosts and warm night temperatures during blossom time contribute to the poor set of Delicious.

Gardner, Merrill and Toenjes (13) have shown that temperature and sunlight during the blooming season influence the set of Delicious. They stated that "The total 'effective' (i. e. above 42° F.) day-degrees in southern Michigan, where setting is characteristically light, for the 7-day period following full bloom is 130-150; for sections where setting

is heavy enough to call for thinning it is 200-250." These investigators used 42° F. as the base point for the various physiological processes influencing fruit setting.

Roberts (33, 34) stated that the structure of the Delicious flower is such as to result in the failure of many flowers being pollinated. He reported in 1945 that "In 1944 it was noted that honeybees collected nectar from Delicious blossoms in 80% of their visits without touching the stigmas. This season the percentage dropped to approximately 50% as the petals were smaller and more cupped, the result being that bees entered the blossom more often from the top." He further stated that bees which gather pollen rather than nectar do not always touch the stigmas due to the fact that the pistils of Delicious are so short. As will be discussed later, the writers' observations were not entirely in agreement with the above.

Frequently, in years when the fruit-set on Delicious has been unsatisfactory for a commercial crop, other diploid varieties such as Jonathan, Grimes Golden, and Rome Beauty growing in the same areas produced at least a fair crop. This circumstance has led some workers to believe that possibly there may be one or more inherent characteristics of the Delicious variety which account for its precarious setting of fruit.

Howlett (22, 25) and Hough (19) suggested that irregularities in the development of the female gametophytes of this variety may play an important part in its fruit setting behavior.

Due to the fact that Delicious has been the leading apple variety, as far as total commercial production is concerned, in the United States since about 1935, the problem regarding its erratic setting deserved further investigation. The grower would undoubtedly welcome additional information as to whether or not this variety might be grown under present cultural methods so as to prove more profitable over more seasons. The extent to which the inherited abnormalities are a factor in the productiveness of this variety, and what can be done to compensate for these has deserved thorough study.

This work was conducted for the purpose of examining the various stages in the development of the embryo sacs in this variety and to evaluate any variations in this development which occur.

LITERATURE REVIEW

A. Abnormalities in the Development of the Megagametophyte

Howlett (20) stated in 1927 that "The failure of many flowers to set fruit in such varieties as Stayman Winesap, Arkansas Black, Rhode Island Greening and Delicious might possibly be due to abnormalities

in their embryo sacs." Again in 1938 Howlett (25) stated that "In triploid varieties a considerable number of the female gametophytes showed degeneration; whereas in the diploid varieties as a whole abortion of the nuclei in the embryo sacs occurred infrequently except in Delicious". About the same time Wanscher (39) reported that in triploids usually only one of the archesporial cells functioned, but if meiosis failed, or if the cells in the tetrad died, another cell which was located above the original megaspore mother cell sometimes took its place. He also found two megaspores becoming twin embryo sacs.

In one of the more recent studies on the life history of several apple varieties, Hough (19) has revealed interesting findings. With Delicious in particular, he stated in 1947 that "In the development of the ovules of Delicious, the most frequent abnormality was either a tardy initiation of the megaspore mother cell or a slower rate of development of the megaspores and the embryo sac. Such retarded embryo sacs would seldom be expected to develop into fully differentiated eight nucleate embryo sacs in time for fertilization especially if their development continued to be at a slower than normal rate. Other apparently normal embryo sacs broke down soon after the flower opened even though the flower had been pollinated with compatible pollen. Less frequently the megaspores or young embryo sacs collapsed. In every case where the megaspores or embryo sacs failed to develop fully—and in some ovules where the embryo sac appeared to be normal—secondary megaspores or embryo sacs were differentiated in the nucellar axis above the primary megaspores or embryo sac. These secondary embryo sacs, like the retarded primary embryo sacs, would rarely be expected to develop into a mature embryo sac in time for fertilization. The frequent evidence of nuclei in early spireme, synapsis or diakinesis stages in the cells in the nucellar axis indicates that these secondary megaspores were produced through meiotic division". As will be seen the authors' findings substantiate much of this work.

B. Flower Position and Fruit Set

The relationship of the flower position on the cluster base to fruit set has been given little study.

Howlett (20) in 1927 stated that "A large proportion of the vigorous lateral flowers of Stayman Winesap and Arkansas Black are unable to set fruit no matter by what variety pollinated. This expresses itself in the fact that two-thirds or more of the fruits on the tree after the first drop are in the central (terminal) position". In regard to Delicious, Howlett (21) in 1928 reported that the set of laterals on a cluster was depressed by the presence of the terminal flower.

Detjen (9) stated in 1929 that the terminal flower on the cluster base being "better constituted and better situated" usually gave a higher percent set over the laterals. He found that the smallest lateral, which is located directly below the terminal, set fruit slightly less often than the other laterals lower on the cluster base.

Howlett (23) summarizes his work with Stayman Winesap in 1931 as follows: "When the terminal was uninjured it depressed the set of the laterals. Only on the more vigorous clusters did laterals set in competition with the terminal. The greater proportion of the laterals which set in competition with the terminal were those in the axils of subtending leaves, rather than those in the axils of bracts. When the terminal was absent or injured the laterals set a higher percentage than otherwise. With the terminal flower eliminated from the competition the laterals in the axils of subtending leaves still set a higher percentage than those in the axils of bracts. The smallest lateral, usually the one adjacent to the terminal and the last to open, failed to set, either alone or under competitive conditions, as satisfactorily as the larger laterals in the axils of a bract. These experiments indicate that the foliage leaf subtending a lateral is a factor in setting. It is not, however, of primary importance."

In regard to Delicious, Howlett (24) in 1932 also found that laterals not situated in the axil of a leaf failed to set as well as those lateral flowers with subtending leaves.

In 1938 Howlett (25) reported that in the variety Arkansas, there was three times as much female gametophyte degeneration in the terminal flowers as in the laterals.

C. Fruit Setting on Weak and Strong Spurs

Heinicke (18) in showing that from 1/6 to 1/3 of the flower-bearing spurs finally set fruit emphasized the importance of vigor of the spur as a factor in fruit setting.

Howlett (21) in working with Delicious observed that the highest percentage of fruit-bearing clusters as well as the greatest number of fruits per cluster have been obtained in locations on the tree where the vigor was the greatest. He found that only in the very tops of heavily pruned Delicious trees have there been found an appreciable number of clusters surviving the first drop with three or four fruits.

Dorsey (10) found that ovules enlarged slowly in flowers which were weak and exhibited a yellowish cast as early as two or three days after full bloom. Also, he noticed in York Imperial flowers on weak spurs that receptivity of the embryo sac does not always seem to occur; or, if it does, its duration is limited to two days at most.

Cooper (8) concluded that "any factor contributing to normal vigor of the bearing tree will increase the set of fruit, and any factor which inhibits vigor will reduce the set of fruit".

D. Stigma Receptivity

In 1901 Goff (14) reported that he believed a moist atmosphere retards the maturity of the pistils.

Haber (17) noticed that the pistils in many of the Delicious blossoms were defective. He found that in many cases the pistils were curled instead of upright, and instead of having normal stigmas of a pale green color, the stigmas were sometimes either red or brown.

In 1922 Beaumont and Knight (2) found that the stigmas of various varieties have different stimulating effects on pollen tube growth in a germinating medium. They stated that stigmas of McIntosh or Northwestern Greening stimulate tubes to longer growth in a given period of time than do stigmas of Delicious.

Boyd and Latimer (4) reporting on the relation of weather to pollination in 1934, stated, "Bright sunshine and low humidity at the time of and immediately following pollination is more detrimental than beneficial". They considered failure to obtain a set of fruit in times of cloudy weather occurred only when such weather was accompanied by an extended period of low temperatures since higher temperature under cloudy skies gave exactly opposite results. They further stated that "It should be emphasized that these conclusions relate only to the effect of weather on fruit setting itself and not to its effect on bee activity". The writers have frequently observed stigmas of the Delicious variety which appeared pinkish and brownish within 24 to 48 hours after anthesis.

MATERIALS AND METHODS

A. Collections

The material collected for cytological examination in this study was obtained from several sources as seen in Table 1.

TABLE 1.—Source of Material

Orchard	Location	Number of flowers and fruits collected		
		1946	1947	1948
Ohio State University	Columbus, Ohio	663	189	190
Harry Lutz	Carroll, Ohio	81	——	——
Richards Brothers & Sons	Thurman, Ohio	——	503	——

In addition to the above flowers and fruits, a comparatively small amount of material was used which was collected from the orchards of the Ohio Agricultural Experiment Station at Wooster, Ohio, during the years 1927, 1928, 1936, 1938, 1940, 1941, and 1946.

The trees from which the collections were obtained were in all cases undergoing satisfactory annual growth and appeared to be in good vigor.

In the Ohio State University orchard, the 12 trees from which material was obtained ranged in age from 8 to 30 years old. They were growing under a sod system of culture and received nitrogen in the form of ammonium nitrate at a rate of one to two pounds per tree annually. The time of application was in April. The soil is classified as Miami silt loam. The trees received an annual moderate pruning and have always been given an adequate spray program.

Trees from which material was obtained in the Richards Brothers and Sons Orchard at Thurman, Ohio, were approximately 18 years of age. They were growing under a sod system of culture and received an annual application of a 10-6-4 fertilizer at the rate of about 7 pounds per tree in February and 1½ pounds of nitrate of soda per tree about April 15. The soil is classified as De Kalb silt loam. An annual moderate pruning has been practiced.

B. Cytological Technique

Within an hour or two after the flowers and young fruits were collected, the epidermis of the ovulary along with the pedicel and remaining floral parts was removed by means of a scalpel; and the ovulary was placed immediately in a killing and fixing solution of Belling's Modified Navashin Fluid (28). The material was stored in this fluid until dehydration took place, which, in some cases, amounted to well over a year. Dehydration was accomplished by using the tertiary butyl alcohol method given by Johansen (28).

Infiltration and embedding was also conducted according to Johansen's schedule. The material was placed first in paraffin oil, then into three successive changes of Parowax (Standard Oil Company) and finally into a pure special filtered paraffin wax, (M. P. 56°-58° C.), made by the Coleman and Bell Co.

Sectioning was carried out by means of a Spencer rotary microtome: the thickness of the sections usually being 10 microns.

The slides were stained with Heidenhain's Iron Hematoxylin prepared according to Johansen (28). A total of 1700 serial-section slides were prepared and examined during this study. These slides represent the partial contents of approximately 400 ovularies with a total of over 2000 ovules and their embryo sacs.

C. Procedure

The flowers and young fruits collected were obtained in accordance with the following four experiments:

Experiment 1—Flowers Collected at Anthesis.

Flowers were gathered at the time of anthesis from the various positions on the cluster base. The positions were classified as follows:

a) Terminal—This flower is the first flower of the cluster to open.

b) Laterals in the Axils of Leaves—These flowers are usually the lowest ones on the cluster base and ordinarily the first laterals to open.

c) Smallest Lateral—The smallest lateral in the cluster is almost always located immediately adjacent to the terminal and is usually the smallest and poorest developed flower of the cluster.

d) Laterals With No Subtending Leaf—These lateral flowers are subtended only by a small bract, if any.

Flowers from each of the above described positions were in turn collected as follows:

1) From strong clusters typified by thick cluster bases.

2) From weak clusters having thin diameter cluster bases.

3) From individual trees without reference to diameter of the cluster base.

Clusters were classified as strong or weak according to the system employed by Blake (3).

In view of the material collected, as outlined above, this experiment was expected to yield information relative to the difference in extent of embryo sac degeneration in ovules of flowers taken from different positions on the cluster base and also any differences between the extent of embryo sac degeneration in ovules of flowers on strong and weak spurs. In addition, the cytological examination was expected to reveal any differences in embryo sac degeneration in ovules from flowers on different trees.

Experiment 2—Non-pollinated Flowers Collected at 24-hour Intervals After Anthesis.

This experiment was conducted in order to observe the extent of degeneration and period of viability of embryo sacs of ovules in flowers which were non-pollinated and collected at 24-hour intervals after

anthesis. Selected, representative branches were bagged with cheesecloth several days prior to anthesis. At anthesis, the laterals in the axil of a leaf and a smaller number of terminal flowers were tagged and dated. These tagged flowers were then collected at 24-hour intervals from the second to the sixth day after anthesis.

Experiment 3—Flowers Pollinated at 24-hour Intervals After Anthesis.

In this experiment, bags were also placed on representative branches prior to anthesis. Laterals in the axil of a leaf and terminal flowers were tagged at anthesis and pollinated with Jonathan pollen at 24-hour intervals from anthesis until six days later. These pollinated flowers were, in turn, collected at 24-, 48-, and 72-hour intervals after pollination. Observation of this material was expected to furnish some indication of the length of time subsequent to anthesis that the stigma was receptive, and also the length of time required from pollination to fertilization under the environmental conditions prevalent at the time.

Pollen used in this experiment was gathered several days in advance; air dried and stored in sealed vials until needed. Just prior to use the pollen was tested for germinability on 4 percent cane sugar—2 percent agar (28). Only pollen which showed approximately better than 60 percent germination was used.

Experiment 4—Embryo and Endosperm Development.

Material for the final experiment consisted of young enlarged fruits which were collected as "expected drops" from 2 to 7 weeks after anthesis. These young fruits were, for the most part, from lateral positions on the cluster base, and all developed from open-pollinated flowers. This material was expected to give some indication as to whether or not embryo abortion was a factor in the abscission of young fruits.

D. Weather Conditions

During each of the blooming seasons of 1946, 1947, and 1948 when material was collected, there was a day or two when weather conditions were unfavorable to the extent that signs of frost injury were apparent on the flowers collected. This injury was especially severe in 1946 when the thermometer in the Ohio State University orchard recorded sub-freezing temperatures on six days from the time the terminal flowers opened until full bloom. These temperature records are presented in Table 2.

The 1947 blooming season like that of 1946 had a number of days which were sub-freezing between the anthesis of terminal flowers and full bloom (Table 3). In addition to freezing temperatures, the 1947 blooming season in the vicinity of Columbus, Ohio, was far from ideal from the pollination standpoint. Most of the days during bloom were too cold, wet, or windy for bee flight.

TABLE 2.—Weather Record of Ohio State University Orchard During Blooming and Early Fruit Setting Period—1946

Date	Stage of bud development	Weather observations	Temperature degrees F. Max.	Min.	Rainfall Inches
April					
1		Cloudy, windy	62	32	.01
2		Partly cloudy	74	44	.16
3	Pre-pink	Partly cloudy	73	42	Trace
4	Pink	Partly cloudy, windy	63	43	.00
5	Pink	Clear, partly cloudy	61	38	.00
6	Pink	Cloudy, rain	54	38	.26
7	Pink	Clear	59	27	.00
8	Pink	Cloudy	60	34	.08
9	Full pink	Clear	55	29	.00
10	Terminals opening	Clear	62	27	.00
11	Terminals opening	Clear	50	31	.25
12	Terminals open	Clear	54	26	.05
13	Terminals open	Cloudy, rain	62	38	.02
14	Laterals opening	Mostly clear	72	54	.00
15	Laterals opening	Rain	62	29	.32
16	Laterals open	Clear	52	24	.00
17	Laterals open	Clear	63	35	.00
18	Full bloom	Clear to partly cloudy	68	29	Trace
19	Full bloom	Clear to partly cloudy	66	44	Trace
20	Full bloom	Clear	62	42	.00
21	Petals dropping	Clear	74	38	.00
22	Petals ⅓ off	Clear to partly cloudy	84	45	.00
23	Petals ⅓ off	Clear to partly cloudy, showers	87	51	.30
24	Petals off	Partly cloudy	84	58	.00
25		Clear to partly cloudy	66	39	Trace
26		Partly cloudy, windy	75	36	.01
27		Partly cloudy	56	36	.00
28		Partly cloudy	59	22	.18
29		Partly cloudy	63	45	.08
30		Clear to partly cloudy	69	37	.00

The occurrence of frost also interfered with the collection of satisfactory flowers and fruits during the 1947 blooming season at the Richards Brothers and Sons Orchard near Thurman, Ohio, in the southwestern part of the state, as seen in Table 4. However, from the standpoint of bee activity and pollination, the season was more favorable in southeastern Ohio than in the vicinity of Columbus.

The 1948 season was the most favorable at the University Orchard as far as pollination and fruit set were concerned. Only a small degree of frost injury occurred during the blooming season. The days were mostly warm, clear, and quiet during the period of bloom.

TABLE 3.—Weather Record of Ohio State University Orchard During Blooming and Early Fruit Setting Period—1947

Date	Stage of bud development	Weather observations	Temperature degrees F. Max.	Min.	Rainfall Inches
May 6	Terminals opening	Partly cloudy, little wind, too cool for bees	61	41	Trace
7	Terminals opening	Partly cloudy, very windy, no bee activity	50	41	.27
8	Terminals open	Clear in a. m., cloudy in p. m., some wind, too cool for bees	45	30	Trace
9	Terminals open	Clear to partly cloudy, only a little bee activity	47	27	.00
10	Terminals open	Clear, only few bees	61	26	.00
11	Laterals opening	Clear and warm	73	32	.00
12	Laterals open	Clear, some wind	79	43	.00
13	Full bloom	Cloudy, rain	71	58	.86
14	Full bloom	Cloudy, poor bee activity	63	57	.00
15	Petals dropping	Partly cloudy to clear, fair bee activity	79	52	.00
16	Petals ½ off	Cloudy in a. m., partly cloudy to clear in p. m. several showers, bees working between showers	77	62	.11
17	Petals off	Cloudy, showers all p. m. no bees	73	61	1.44
18	"Drops" are falling	Clear to partly cloudy	77	63	.02
19		Clear to cloudy, sultry, showers	78	58	.01
20		Partly cloudy	78	61	.02
21		Rain until 3 p. m., then partly cloudy	68	53	.59
22		Clear to partly cloudy	73	43	.00
23		Clear	80	49	.00
24		Cloudy, quiet	82	59	.12

PRESENTATION OF DATA

A. Experiment I—Flowers Collected at Anthesis

The frequency and types of abnormalities which were observed in the examination of the embryo sacs of flowers collected at anthesis may be seen in detail in Table 6 and summarized in Table 7. A total of 12.7 percent of the ovules were delayed in development at the time of anthesis. A few ovules were found among the 618 examined from

TABLE 4.—Weather Record of Richards Brothers and Sons Orchard During Bloom and Early Fruit Setting Period, 1947

Date	Stage of bud development	Weather observations	Temperature degrees F. Max.	Min.
April				
23		Clear, little wind	85	60
24		Cloudy, little rain	70	58
25	Terminals opening	Cool, rain	60	40
26	Terminals open	Quiet	63	43
27	Laterals opening	Cloudy in p. m. with rain and turned cold	72	46
28	Laterals open	Clear, warm in p. m.	66	30
29	Full bloom	Warmed up rapidly to 2 p. m. then cloudy with rain at 5 p. m.	81	39
30		Rain all day	61	56
May				
1	Petals falling	Blossoms wet until noon. Hard showers and wind in p. m.	80	54
2		Cloudy, showers	71	48
3		Showers	63	46
4	Petals off	Showers in late p. m.	74	50
8			56	29
9			58	29
10			67	30

flowers collected at anthesis that apparently contained no megaspore mother cell, megaspore or embryo sac. These particular ovules were mostly small in size and certainly delayed in development. All of the nucellar cells in these ovules were quite similar in appearance and none contained what could be called a prominent nucleus.

Several ovules examined in the material collected at anthesis contained only a megaspore mother cell. In such an ovule (Figure 2) a deeply stained cell having a large prominent nucleus was presumed to be a megaspore mother cell as no degenerating megaspores were to be found adjacent to it.

Of the ovules examined which showed delayed development at anthesis, 6.5 percent contained only megaspores. Ovules were found which contained megaspores in various stages of development. One ovule (Figure 3) was observed in which the micropylar megaspore had degenerated while the other three still appeared to be functional.

TABLE 5.—Weather Record of Ohio State University Orchard During Blooming and Early Fruit Setting Period, 1948

Date	Stage of bud development	Weather observations	Temperature degrees F. Max.	Min.	Rainfall Inches
April					
19	Full pink, some terminals opening	Clear, windy	75	50	.00
20	Terminals open	Cloudy, windy	79	53	Trace
21	Terminals open	Cloudy, very chilly	56	42	.00
22	Laterals opening	Clear	70	32	.00
23	Laterals open	Partly cloudy	74	48	Trace
24	Laterals open	Clear	84	54	Trace
25	Full bloom	Clear	89	49	.00
26	Petals dropping	Clear	89	52	.00
27	Petals ¾ off	Clear, thunder showers	79	59	.51
28	Petals off	Cloudy, rain	57	46	.08
29		Clear to partly cloudy	64	40	.00
30	Few "drops'	Clear	67	34	.00
May					
1	Terminals dropping	Cloudy, light rain	67	46	.01
2	Terminals dropping	Rain in a. m., partly cloudy in p. m.	71	40	1.22
3	Laterals dropping	Clear	70	46	.00
4	Laterals dropping	Cloudy, rain	61	40	.39
5	Laterals dropping	Clear	73	42	.00
6	Laterals dropping	Cloudy, rain	69	41	.49

In a number of the ovules examined, more than one tetrad of megaspores was present. In such cases, one tetrad of megaspores always appeared more advanced in development. Where two sets of megaspores were observed in the same ovule, it was found that sometimes they were orientated end to end in the nucellar axis, while in the other ovules the two sets of megaspores were situated side by side.

Several ovules were observed in which a megaspore other than the chalazal one appeared to be the functional megaspore. One of these ovules (Fig. 4) contained a degenerating chalazal megaspore while the megespore directly below it appeared functional. The two megaspores nearest the micropyle had degenerated entirely.

A two-nucleate sac was observed in 1.5 percent of the ovules examined from flowers collected at anthesis. The ovule shown in Figure 5 was obtained from the smallest lateral flower of a cluster and contains a small two-nucleate embryo sac. Other ovules were observed with larger two-nucleate embryo sacs, some of which appeared to be degenerating.

TABLE 6.—Percentage of Ovules with Embryo Sac at Given Stages of Development in Flowers Collected at Anthesis From Different Cluster-Base Positions

Flower position	No. of ovules examined	Megaspore stage	2-Nucleate sac	4-Nucleate sac	8-Nucleate Sac					Ovules with more than one sac
					Not fully differentiated	Fully differentiated	Degenerating egg	Degenerating synergids	Entire sac degen.	
Terminal	223	3.6	0.0	0.5	0.0	76.7	4.0	13.0	3.1	3.1
Lateral in axil of leaf	174	7.5	1.2	1.2	4.6	73.6	2.3	8.1	2.9	2.9
Lateral no leaf	137	5.1	1.5	0.7	10.2	67.2	2.9	6.6	3.7	4.4
Smallest lateral (no leaf)	84	14.3	6.0	1.2	2.4	63.1	1.2	8.3	1.2	1.2
Total and percentages of total	618	6.5	1.5	.8	3.9	71.9	2 9	9.5	2.9	3.1

Approximately 1 percent of the ovules examined at anthesis contained embryo sacs in the four-nucleate stage of development. This stage, as well as the two-nucleate stage, is known to be of comparatively short duration, and the small percentage of sacs found here may be partly accounted for by this fact.

In 3.9 percent of the ovules examined which were from flowers collected at anthesis an eight-nucleate but not fully differentiated embryo sac was present. One of these embryo sacs is seen in Figure 7. An embryo sac at this stage of development at anthesis is only slightly delayed and undoubtedly has a good chance of being fertilized. However, the question would naturally arise as to whether those ovules containing megaspores, two-nucleate or four-nucleate embryo sacs would develop into eight-nucleate sacs in time for fertilization.

The eight-nucleate embryo sac is usually attained in diploid apple varieties just prior to the opening of the flower (16). At anthesis the egg may be differentiated from the synergids and the antipodals have begun to degenerate in some cases. An example of a fully differentiated eight-nucleate embryo sac is shown in Figure 8. In this embryo sac the polar nuclei are in close proximity in the center of the sac while the synergids and egg cell (which is out of focus) are orientated in the typical manner at the micropylar end of the sac. A fully differentiated embryo sac in which the polar nuclei are fused is shown in Figure 9.

In contrast to the ovules delayed in development at anthesis, a total of 15.3 percent (Table 6) of the ovules were premature in their development or showed early degeneration. The egg appeared to be degenerated in 2.9 percent of the embryo sacs. In such ovules, the cytoplasm of the egg appeared shrunken and the nucleus was disorganized (Figure 10).

In 9.5 percent of the ovules one or both of the synergids appeared degenerated, and in 2.9 percent of the ovules the entire embryo sac showed breakdown (Figure 11).

In addition to those ovules which contained embryo sacs delayed in development or prematurely degenerated, 3.1 percent of the ovules examined in this experiment contained more than one embryo sac.

Only in 71.9 percent of the ovules examined was there a fully differentiated eight-nucleate sac at the time of anthesis, indicating that almost a third could be classified as "abnormal" to some extent.

A more precise picture of the results of this experiment is possibly obtained from an examination of Table 7, which is a partial condensation of Table 6.

Embryo sacs that were delayed in development ranged from 4 percent in the case of terminal flowers to 24 percent in the smallest laterals

TABLE 7.—Percentage of Ovules from Flowers Collected at Anthesis with Stage of Development of the Embryo Sac

Flower position	Embryo-Sac not fully differentiated	Fully differentiated 8-Nucleate sac	Prematurely degenerating sac	More than one sac
Terminal	4	77	16	3
Lateral in axil of leaf	14	74	9	3
Lateral no leaf	18	67	11	4
Smallest lateral (no leaf)	24	63	12	1
Total	13	72	12	3

with no subtending leaf. For the entire sample the amount of embryo sacs that were delayed in development was 13 percent. On the other side of the picture, with respect to degenerating embryo sacs, the difference based on cluster base position was considerably less ranging from 16 percent in the case of terminal flowers to 9 percent in laterals in the axil of a leaf. In addition, further inspection of the table reveals that flowers with normal embryo sacs decreased from 77 percent in the case of terminal flowers; 74 percent in lateral flowers in the axil of a leaf; 67 percent in laterals with no leaf; to 63 percent in the smallest laterals with no subtending leaf.

Data for the second part of this experiment, concerning flowers collected from strong and weak spurs at anthesis, are presented in Table 8. The extent of embryo sac immaturity and degeneration was again found to be quite variable. Flowers from strong spurs as well as weak spurs contained ovules with embryo sacs in all stages of development. A rather large percentage of the ovules in flowers from strong spurs, namely 14 percent contained megaspores; while in flowers from weak spurs, only 4 percent of the ovules contained megaspores at anthesis. Probably little significance should be placed on these percentages as the sample was composed of ovules from flowers from all cluster base positions and the number of ovules examined was not as large as desired. However, the general over-all picture obtained from Table 8 is undoubtedly worthy of evaluation. It indicates that, generally speaking, there is less difference between the condition of embryo sacs from flowers of strong and weak spurs than expected.

The stage of development of embryo sacs in flowers at anthesis from strong and weak spurs is also recorded in Table 9 which is a partial condensation of Table 8. In flowers from strong spurs 17 percent of the ovules contained immature embryo sacs while those from weak spurs contained 10 percent. However, in regard to prematurely degenerating

TABLE 8.—Percentage of Ovules with Embryo Sac at Given Stages of Development in Flowers Collected at Anthesis from Strong and Weak Spurs

Flowers obtained from	Number ovules examined	Megaspore stage	2-Nucleate sac	4-Nucleate sac	8-Nucleate Sac					More than one sac
					Not differentiated	Fully differentiated	Degenerating egg	Degenerating synergids	Entire sac degenerating	
Strong spurs	86	14.0	2.3	1.2	0.0	75.6	1.2	3.5	0.0	5.8
Weak spurs	101	4.0	1.0	0.0	5.0	78.2	4.0	5.0	3.0	1.0

TABLE 9.—Percentage of Ovules from Flowers Collected at Anthesis with Stage of Development of Embryo Sac

Flowers obtained from	Ovules having immature embryo sac	Fully differentiated 8-Nucleate sac	Prematurely degenerating sac	More than one sac
Strong spurs	17	76	1	6
Weak spurs	10	78	11	1

sacs the figures were 1 percent versus 11 percent, respectively. In spite of these differences in extremes of embryo sacs development the percentage of fully differentiated eight-nucleate embryo sacs was 76 percent in the case of flowers from strong spurs and 78 percent from weak spurs, an insignificant difference.

A final part of this experiment consisted of observing the extent of immaturity and degeneration in embryo sacs of flowers which were collected from different individual trees. Due to considerable frost injury (Figure 25) during the period of bloom, the number of ovules free from injury in flowers from any one tree was rather limited. The data presented in Table 10 include the examination of 506 uninjured ovules taken from three trees. Although there appeared to be a considerable difference in the percentage of fully differentiated embryo sacs in flowers from comparable positions on the cluster base of different trees, the percentage of fully differentiated embryo sacs from flowers from all cluster base positions taken collectively was very small, namely 71 percent, 73 percent and 74 percent for the three trees considered, and thereby insignificant as far as the difference between individual trees is concerned.

TABLE 10.—Percentage of Fully Differentiated 8-Nucleate Embryo Sacs in Ovules from Flowers Collected at Anthesis from Different Cluster-base Positions on Individual Trees

Tree	Number flowers and (ovules) in sample	Percentage of Ovules with Fully Differentiated Embryo Sacs in Flowers from Various Positions on the Cluster Base				
		Terminal	Lateral in axil of leaf	Lateral no leaf	Smallest lateral (no leaf)	Totals
3	25 (230)	71	73	81	59	71
13	11 (95)	78	65	61	80	73
20	21 (181)	86	76	58	62	74
Total	57 (506)	79	72	69	65	—

TABLE 11.—Percentage of Ovules with Embryo Sac Stage of Development in Bagged Flowers Collected at 24-Hour Intervals After Anthesis

Time after anthesis	Number ovules examined	Percentage of Ovules With								
					8-Nucleate Sac					More than one sac
		Mega-spore stage	2-Nucleate sac	4-Nucleate sac	Not differen-tiated	Fully differen-tiated	Degen-erating egg	Degen-erating syn-ergids	Entire sac degen-erating	
48 hours	42	0.0	0.0	2.4	2.4	73.8	2.4	16.7	0.0	2.4
72 hours	20	0.0	0.0	0.0	0.0	70.0	10.0	20 0	0.0	15.0
96 hours	39	0.0	0.0	0.0	0.0	41.0	7.7	48.7	2.6	2.6
120 hours	31	0.0	0.0	0.0	0 0	19 4	6.5	48.4	0.0	32.3
144 hours	22	0.0	0.0	0.0	0.0	13 6	13.6	77 3	0.0	4.6
2 weeks	9	0.0	0.0	0.0	0.0	0.0	11.1	11.1	89.9	0.0

B. **Experiment II—Non-pollinated Flowers Collected at 24-hour Intervals After Anthesis.**

The data relative to the occurrence of immature and degenerating embryo sacs in ovules of flowers which received no pollination, and were collected at 24-hour intervals beginning at 48 hours after anthesis, are given in Table 11. All of the flowers in this experiment were either from the terminal position on the cluster base or laterals in the axil of a leaf. Immature embryo sacs were observed in those ovules from flowers which were collected at 48 hours after anthesis (Figure 6). The percentage of embryo sacs with degenerating nuclei was 26 percent in flowers collected 48 hours after anthesis. At 72 hours after anthesis the percentage of degenerating embryo sacs rose to 30 percent and then

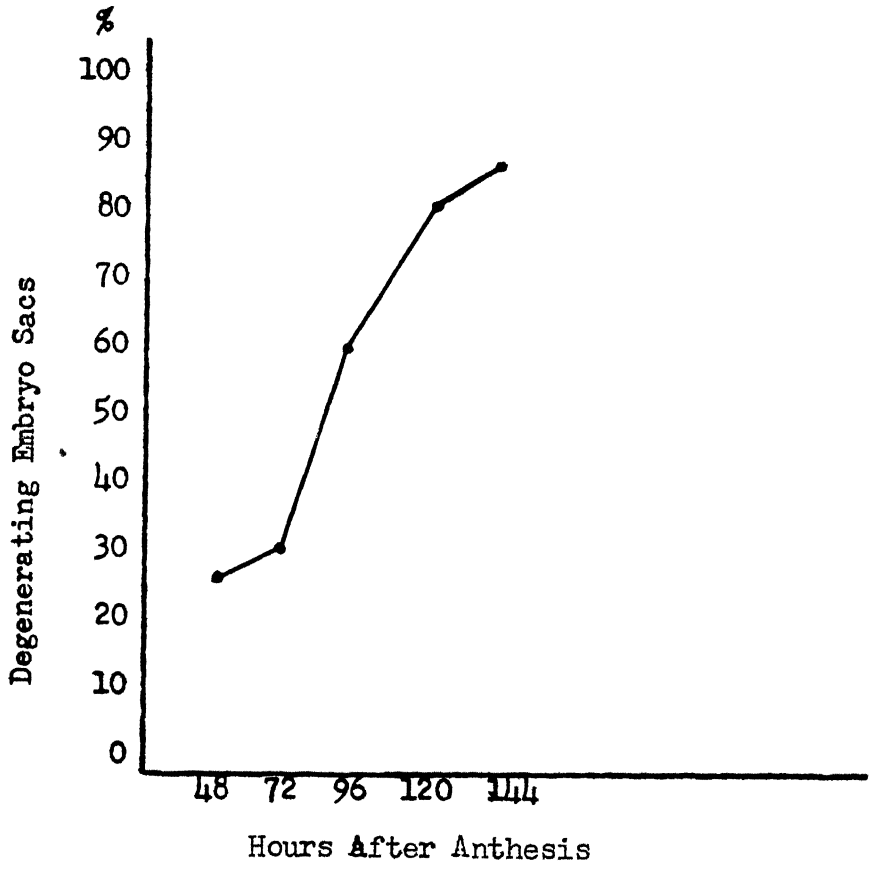

Fig. 1.—This shows graphically the degeneration of embryo sacs after anthesis. An outstanding increase after 72 hours is noted.

increased rapidly until at 96, 120 and 144 hours after anthesis the percentages were 59 percent, 81 percent, and 87 percent, respectively. This increase is depicted graphically in Figure 1 and indicates an outstanding increase after 72 hours. More conclusive data concerning multiple embryo sacs are presented below.

C. Experiment III—Flowers Pollinated at 24-hour Intervals After Anthesis.

This experiment, which consisted of the hand pollination of flowers at 24-hour intervals after anthesis and the subsequent collection of the flowers 48 hours or more after pollination, was not only limited by frosts, but also by rain and cold weather which resulted in poor pollen germinability and stigma receptivity.

One objective of this experiment was the acquisition of data which might furnish an indication as to the period of receptivity of the stigmas as well as functionability of the embryo sacs subsequent to anthesis. The occurrence of fertilization, as indicated by the presence of endosperm nuclei, zygote or embryo, was the basis for measuring stigma receptivity. The percentage of ovules with evidence of the presence of an endosperm, zygote or embryo is given in Table 12.

Flowers pollinated 48 hours after anthesis contained ovules in which fertilization appeared to have occurred in approximately 10 percent of the cases. The occurrence of fertilization decreased markedly in ovules of flowers which had been open more than 48 hours prior to pollination. No indications of fertilization were to be found in ovules of flowers which were pollinated 120 hours after anthesis. The low percentage of fertilization observed in ovules from flowers pollinated 24 hours after anthesis is unexplainable.

From the data presented in Table 14 it appears that usually more than 48 hours were required subsequent to pollination before indications of fertilization were evident in the embryo sac. The ovule in

TABLE 12.—Percentage of Ovules Pollinated at 24-hour Intervals After Anthesis Containing Endosperm Nuclei, Zygote or Embryo

Hours pollinated after anthesis	Number of ovules examined	Percentage with	
		Endosperm	Zygote or Embryo
24	97	5	1
48	98	10	12
72	45	2	0
96	66	3	3
120	29	0	0

Figure 18 which was from a flower collected two days after pollination apparently, based on its size and the presence of more than one nucleolus within the nucleus, contains a fertilized egg. The small dark spot beside the fused polar nuclei is presumably a sperm nucleus. The ovule in Figure 19 contains an embryo sac which apparently was fertilized even though pollination was delayed until four days after anthesis. In the majority of flowers of the Delicious apple variety, which the writers examined, the stigmas appeared to have lost their receptivity by this time. In five other ovules in addition to the one shown in Figure 19 single fertilization of the embryo sac was evident.

The developing seed pictured in Figure 20 was obtained from a young fruit which originated from a flower that was pollinated 48 hours after anthesis and collected three days after pollination. The results of double fertilization are apparent from the presence of free endosperm nuclei and zygote. A small embryo and a degenerated synergid are to be seen in Figure 21 which was observed in a seed from a young fruit which developed from a flower pollinated two days after anthesis and collected five days after pollination.

To supplement the cytological observations of the occurrence of fertilization, a number of flowers were left on the trees after hand cross-pollination in order to check the percentage developing into fruits. Here, too, frost and cool wet weather reduced the set considerably. The data are given in Table 13. The small fruits were collected four to five weeks after anthesis.

The percentage of flowers, cross-pollinated by hand at anthesis, which set fruit was 61 percent while that of flowers pollinated 24 hours after pollination was 27 percent. When pollination was delayed until 48 hours after anthesis, only 11 percent of the flowers set fruit. Moreover, only 3 percent of the flowers set fruit when pollination was delayed until 72 hours after anthesis. The decrease in percentage of fruit set with increasing periods of time between anthesis and pollination may be

TABLE 13.—Set of Fruit From Flowers Pollinated at 24-hour Intervals After Anthesis

Time of pollination	Number of flowers pollinated	Percent of flowers setting fruit
Anthesis	56	61
24 hours after	65	27
48 hours after	18	11
72 hours after	37	3

related to the increase in embryo sac degeneration with hours subsequent to anthesis. Also these data showing greatly reduced set after 48 hours agree closely with reduced fertilization shown in Table 12.

In order to obtain data pertaining to the length of time required from pollination to fertilization under the particular environmental conditions present, flowers were collected at 24-hour intervals after pollination and the embryo sacs examined. These data are presented in Table 14.

Only a small percentage of the ovules had evidence of fertilization prior to 72 hours subsequent to pollination. This data when considered in relation to the data which showed the high percentage of degenerating embryo sacs at 72 hours subsequent to anthesis (Table 11) explains to some degree the cause of poor fruit setting in Delicious.

D. Experiment IV—Embryo and Endosperm Development

The data obtained from an examination of ovules in young fruits which were collected as "expected drops" revealed that 80 percent of the ovules had collapsed embryo sacs indicating lack of fertilization. In the remaining 20 percent of the ovules examined endosperm nuclei were observed in all cases while only 17 percent of these ovules contained an embryo. Most of the embryo sacs which had collapsed apparently had not been fertilized as no zygote, embryo or endosperm could be detected. Examples of ovules contained in such fruits are to be seen in the following photomicrographs (Figures 22, 23, 24). These fruits presumably would have abscissed during the "second drop".

E. Some Additional "Abnormalities" Observed in the Ovules of Delicious.

Polar Nuclei

The polar nuclei had fused in 16 percent of the ovules from flowers which were collected at anthesis (Table 15). In flowers collected at 24-hour intervals after anthesis, the occurrence of fused polar nuclei

TABLE 14.—Percentage of Ovules Collected at 24-hour Intervals After Pollination Containing Endosperm Nuclei, Zygote or Embryo

Hours collected after pollination	Number of ovules examined	Percentage with	
		Endosperm	Zygote or Embryo
24	126	0	1
48	233	1	2
72	108	10	9
120	10	40	30

increased to 59 percent about 96 hours after anthesis. There was apparently no decided increase in fused nuclei in pollinated flowers over non-pollinated ones (Table 15).

TABLE 15.—Percentage of Fused Polar Nuclei in Ovules of Non-pollinated and Pollinated Flowers

Time of collection	Non-pollinated Flowers		Pollinated Flowers	
	Number ovules observed	Percentage of fused nuclei	Number ovules observed	Percentage of fused nuclei
Anthesis	618	16	—	—
24 hours later	10	0	—	—
48 hours later	42	36	34	21
72 hours later	20	35	97	42
96 hours later	39	59	92	43
120 hours later	31	39	88	56
144 hours later	22	55	82	54
168 hours later	—	—	56	55
192 hours later	—	—	24	50

Multiple Embryo Sacs

The frequency of the occurrence of multiple embryo sacs within an ovule was somewhat surprising (Table 16). Ovules from flowers collected at anthesis contained multiple embryo sacs in 3 percent of the cases, while this frequency increased in ovules from flowers collected at additional daily intervals subsequent to anthesis to 43 percent at 216 hours after anthesis. In most cases where the embryo sacs were orientated end to end in the nucellar axis, the lower or micropylar sac was the further advanced in development.

TABLE 16.—Percentage of Ovules from Flowers Collected at Anthesis and Subsequent 24-hour Periods Which Contained Multiple Embryo Sacs

Time of collection	Number of ovules observed	Multiple embryo sacs Percent
Anthesis	618	3
24 hours after	10	1
48 hours after	58	5
72 hours after	117	13
96 hours after	131	6
120 hours after	129	16
144 hours after	112	16
168 hours after	60	10
192 hours after	24	33
216 hours after	7	43
Total	1266	8

Many ovules actually only had one embryo sac, but in addition contained a megaspore mother cell or megaspores. The ovule illustrated in Figure 12 contains a fully differentiated eight-nucleate embryo sac with a megaspore mother cell above it. An ovule is pictured in Figure 13 having an embryo sac with a set of four megaspores above it. The chalazal appeared functional and was subtended by three degenerated megaspores. Another ovule containing a sac with fused polar nuclei with a megaspore above is seen in Figure 14.

In addition to multiple embryo sacs located end to end in the nucellar axis, several were observed in which embryo sacs were side by side (Figure 15). It is probable that these two embryo sacs developed from megaspores which developed from the same megaspore mother cell.

An interesting ovule containing two embryo sacs of extremely different size is shown in Figure 16. The large sac appears to be in the telophase stage of the division of the primary endosperm nucleus.

Although not as frequent in occurrence as twin embryo sacs, several ovules were found to contain three embryo sacs. In Figure 17 the micropylar embryo sac is eight-nucleate with only the shrunken, degenerating egg faintly discernible. Above the eight-nucleate sac is a four and a two-nucleate sac side by side.

DISCUSSION

A. Stage of Development of the Megagametophyte at Anthesis

The effect upon fruit set of the immature condition of the ovule as shown by the presence of megaspores and immature embryo sacs at anthesis, as observed in Delicious in this work and in other varieties by von Veh (38) and Schneider (36) cannot be definitely stated. Presumably, those embryo sacs which were in the four- to eight-nucleate stage at anthesis (Figures 6, 7) might have reached the fully differentiated eight-nucleate stage by the time the male gametes entered the micropyle of the ovule. However, in those ovules which contained a megaspore mother cell (Figure 2) or megaspores (Figure 3) at anthesis it is difficult to conceive of fertilization occurring. Although no data were given here-in which directly support the above assumption, the rather rapid loss of receptivity of the stigmas subsequent to 48 hours after anthesis (Table 12) would indicate that by the time these delayed embryo sacs had reached the fully differentiated eight-nucleate stage the stigmas would no longer be in condition to facilitate pollen germination. Whether or not any male gametes that had reached the micropyle before stigmatic breakdown occurred would remain functional

until the eight-nucleate embryo sac stage had been attained is not known. Hough (19) showed that many embryo sacs in Delicious were not fully differentiated, hence not capable of being fertilized by the time the pollen tubes reached the micropyle. Although the extent to which this factor of immaturity of the megagametophyte may influence fruit set is not definitely known, one can not entirely ignore this as one of the factors influencing set since 13 percent (Table 6) of the ovules examined at anthesis contained immature embryo sacs.

In regard to premature degeneration of the megagametophyte in ovules from flowers collected at anthesis, the effect upon fruit set may be more definitely stated. Approximately 15 percent (Table 6) of the ovules examined at anthesis contained embryo sacs in which degeneration was occurring or had taken place. This fact was also pointed out by Hough (19) who showed that apparently normal embryo sacs collapsed soon after the flowers opened and less frequently megaspores or partially developed embryo sacs broke down.

Degenerating egg cells (Figure 10) were found to be present in 3 percent of the ovules at anthesis. Although this percentage is rather small, one must not overlook the fact that this figure represents only the observable or cytological degeneration. It is entirely conceivable that a greater percentage of the ovules were undergoing some degree of physiological degeneration before it could be observed cytologically.

The egg cell was only one component of the embryo sac in which degeneration was observed at anthesis. In a similar percentage of ovules the entire embryo sac had degenerated (Figure 11). Moreover, in 9.5 percent of the embryo sacs one or both synergids were breaking down. Although the presence of degenerating synergids would not necessarily indicate that fertilization was thereby prevented, nevertheless, it indicated that the embryo sac had begun to degenerate prematurely. Bryant (7) believed that too much importance should not be given to the condition of the synergids.

From the data given above concerning the percentage of immature and degenerating embryo sacs in flowers collected at anthesis it may be seen that approximately 30 percent of the ovules, or three ovules out of ten, are so affected. Therefore, in years when weather conditions are unfavorable for pollination and fertilization, this percentage of immature and degenerating embryo sacs may play an increasingly important part in the eventual fruit set obtained.

The occurrence of immature and prematurely degenerating embryo sacs might be attributed to the nutritional conditions within the tree, spur, or individual flower. However, Howlett (21) suggested the probability of an inherent factor with its expression as abnormalities in

the megagametophyte in addition to inadequate vigor and pollination as being in part responsible for the light setting in Delicious. The data previously presented in regard to the high percentage of degenerating embryo sacs at anthesis tend to substantiate this opinion. For example, the percentage of fully differentiated eight-nucleate embryo sacs was as great in flowers from weak spurs as from strong spurs (Table 9) indicating that factors other than spur vigor were operative.

Within the individual flower cluster, whether from a strong or weak spur, the flowers in the various positions on the cluster base showed considerable difference in the percentage of fully differentiated embryo sacs (Table 7). Terminal flowers contained the highest percentage of fully differentiated eight-nucleate sacs at anthesis while laterals with and without a subtending leaf were next in order, respectively. The smallest lateral with no leaf was in last place with only 63 percent of the embryo sacs being fully differentiated. This observed cytological difference concerning percentage of abnormalities in embryo sacs from flowers in different cluster base positions substantiates observed fruit setting in comparable flower positions as reported by several investigators (9, 20, 21, 23, 24). Terminal flowers have given a higher percentage of fruit set than lateral flowers, and the smallest lateral very seldom sets fruit. Detjen (9) stated that "The central flower of the cluster base, being better constituted and better situated, generally gives a high percent set over the laterals". This flower-position difference might be governed by some limiting environmental factor. Howlett (25) has pointed out that certain unfavorable environmental factors such as low temperature during bloom and deficiency of nitrogen or other minerals and water might influence the development of the embryo sac. Thus, it appears from the data previously mentioned that the development of the embryo sac is apparently dependent to a greater extent upon the location of the flower within the cluster than by the vigor of the spur as a whole. In most cases, the terminal flower contained more fully developed embryo sacs at anthesis than did the small lateral flowers. Terminal flowers from even the weakest spurs averaged more fully differentiated embryo sacs than did the smallest laterals from strong spurs.

Flowers collected from trees growing in several locations, all apparently in good vigor, showed some difference in the percentage of fully differeniated embryo sacs at anthesis when the individual flower positions were compared (Table 10). However, the differences in the percentage of mature sacs when all flower positions were taken collectively were not significant. The amount of fully differentiated embryo sacs from several trees ranged from 71 percent to 74 percent. Any

differences, therefore, in the nutritional status of the individual trees were not clearly expressed in the development of the embryo sac. In all of the trees there was considerable immaturity and early degeneration of the embryo sacs.

Most certainly the importance of the nutritional status or vigor of the tree and spur upon the development of the embryo sac and consequent fruit set is not to be minimized. However, it would appear from the data presented herein that there is another factor which is inherent in this variety which plays an important role in embryo sac development and subsequent fruit set. This is indicated by the common occurrence of immature and degenerating embryo sacs at anthesis from trees in various locations, from spurs of divergent vigor, and from the different flower positions.

It is of further interest to note the percentage of fused polar nuclei which were observed in embryo sacs collected at anthesis. This amounted to 16 percent (Table 15). Schneider (36) reported that approximately 50 percent of the embryo sacs in Jonathan contained fused polar nuclei just after anthesis. In this respect, Delicious again would appear to be somewhat delayed in embryo sac development as far as comparison with Jonathan is concerned.

B. Megagametophyte Viability and Time Required from Pollination to Fertilization

The material examined to determine megagametophyte viability not only revealed considerable degeneration in the embryo sacs at anthesis but also subsequently (Table 11). It has been shown by Howlett (25) that in diploid apple varieties, as a whole, abortion of the nuclei in the embryo sac occurred infrequently except in Delicious. Early breakdown of the embryo sac after anthesis has also been reported by Hough (19) in this variety.

One eventually would expect to observe degeneration of embryo sacs in non-pollinated flowers. However, when this breakdown increased rapidly as early as three days after anthesis (Fig. 1), it would seem that the opportunity for fertilization to occur and consequent setting of fruit might be considerably reduced. This assumption is given added weight by the data (Table 14) which indicate that only a small percentage of the ovules were fertilized within 48 hours after pollination. The rate of pollen tube growth in Delicious is apparently not especially slow judging from the data of other investigators. Bryant (7) first found zygotes five days after pollination in the embryo sacs of both McIntosh × Delicious crosses and open pollinated McIntosh flowers. A fertilized egg was first observed in Jonathan by Schneider

(36) four days after pollination while Knight (29) found that pollen tubes traversed the length of the style in Rome Beauty × Jonathan in 48 hours. The importance of these two factors—early degeneration of the embryo sac nuclei and time required from pollination to fertilization—upon fruit set is undoubtedly of considerable importance in the Delicious apple.

It is suggested that the early degeneration of the embryo sac nuclei subsequent to anthesis is also the expression of an inherent factor in this variety. The high percentage of degeneration observed in this material, which was collected from trees apparently in good vigor, is more comparable to that found in triploid varieties rather than in other diploid varieties. Therefore, this additional degeneration of the embryo sacs subsequent to anthesis would undoubtedly contribute to the reduction of the fruit set.

C. Stigma Receptivity

The presence of pinkish stigmas in flowers of the Delicious apple at anthesis and subsequently, has been reported by Howlett (26) and Haber (17). The senior writer has also observed the occurrence of pinkish and brownish colored stigmas in flowers of Delicious within 24 hours after anthesis. This condition appeared to be more common if the weather was cool and damp when the flowers opened. Presumably the pink color of the stigma was the first stage in the loss of receptivity. Goff (14) believed that a moist atmosphere retarded the maturity of the pistils. Data presented (Table 12) substantiates the rapid loss of receptivity in stigmas of this variety. The percentage of fertilization dropped greatly in flowers in which pollination was delayed more than 48 hours after anthesis. It is suggested that this factor of stigma receptivity likewise plays in important role in fruit set in Delicious.

Another related point of interest which should be mentioned at this time is the matter of flower structure and its relation to fruit setting which has been reported by several investigators. Roberts (33) reported that honeybees extracted nectar from the blossoms without crawling over the anthers and stigmas. Moreover, he stated that pistils of Delicious were so short that bees, which collected pollen rather than nectar, did not always touch the stigmas. Bayar (1) reported that Delicious pistils averaged less than one millimeter shorter than the stamens, while in Richared, a red strain of Delicious, the pistils were a little longer than the stamens. Forshey (12) reported, however, that there was no significant difference between the length of the pistils and stamens of Delicious or its mutation, Richared. He concluded that the pistil-stamen relationship is apparently not a factor in fruit setting of

Delicious. The writers are of the opinion that flower structure is only of very minor importance in relation to fruit setting in Delicious as honeybees usually crawled over the top of the blossoms and appeared to have touched the stigmas.

D. "Abnormalities" in Megasporogenesis and Megagametogenesis

The number and type of "abnormalities" observed in megasporogenesis and megagametogenesis were considerably more than expected. Many ovules were seen to possess more than one megaspore mother cell. This condition has been found to exist in many apple varieties (15, 19, 36, 37, 39). Hough (19) reported that he found cells in the nucellar axis above the primary megaspores or embryo sac in Delicious ovules which began to function as secondary megaspore mother cells. Secondary megasporocytes were found to be present above degenerating as well as functional embryo sacs (Fig. 12). Some of these megasporocytes developed into megaspores (Figs. 13 and 14) and secondary embryo sacs (Fig. 17). Examples of twin embryo sacs have been reported (15, 19, 32, 36, 37) in other varieties besides Delicious.

Where a secondary megaspore mother cell develops within an ovule in which the primary embryo sac is degenerating it is possible that the secondary megaspore mother cell will continue to function and produce megaspores and eventually produce a secondary embryo sac in which fertilization might take place. Schneider (36) reported that several ovules containing a secondary embryo sac underwent fertilization which would indicate that secondary embryo sacs may be functional. However, the writers have observed that the great majority of secondary embryo sacs attained the eight-nucleate stage several days after anthesis which was undoubtedly too late for fertilization since stigma receptivity had become greatly reduced by this time (Table 12). The occurrence of a greater percentage of multiple embryo sacs in ovules collected after anthesis (Table 16) would indicate that the secondary embryo sacs originate considerably later and their origin may be stimulated by degeneration of the primary embryo sac, but they seem to have developed too late to aid fruit set in Delicious.

Where the primary embryo sac remains functional and fertilization occurs, one would be led to believe that the secondary embryo sac is inhibited from developing further since seldom have ovules containing more than one fertilized embryo sac or apple seeds containing twin embryos been reported in the literature.

The development of secondary megaspore mother cells in the nucellar axis toward the chalazal end of the ovule might be explained by the nutritional gradient in the nucellar axis as suggested by Brink

and Cooper (5). These investigators described a nutritional gradient in the nucellar axis which increased from the micropylar toward the vascular bundles in the chalazal end of the ovule. They suggested that this high nutritional status nearest the chalazal end apparently affords a better supply of nutrients to cells in this area. The authors believe that this may account for development of secondary megaspore mother cells observed in this work since these usually were found nearer the chalazal end in relation to the primary embryo sac.

Another observation of this work concerned the megaspore which became the functional one. The chalazal megaspore in the tetrad usually becomes the functional megaspore. However, it was observed that other megaspores in addition to the chalazal one might also develop further (Figs. 3, 4). This condition has been reported by Hurst (27). In some cases apparently more than one megaspore of the tetrad developed into an embryo sac (Figs. 15, 17). Several investigators (19, 37, 38) have found a similar situation in other apple varieties.

Ovules were also observed in which more than one set of megaspores were present. In these ovules the two megaspore tetrads were either side by side or one set above the other. In the former situation the megaspore mother cells from which the two sets of megaspores developed were apparently located beside one another in the nucellus. In the other example the two megaspore mother cells were differentiated one above the other in the nucellar axis. Where two sets of megaspores were observed the lower set appeared to be undergoing more extensive degeneration than the chalazal quartet. This again is in agreement with the thecry of Brink and Cooper (5). Again in the case of multiple sets of megaspores within an ovule, it is very probable that the secondary set was delayed to such an extent that the resulting embryo sac, if attained, would be too late for fertilization. This likewise would result in decreased fruit set.

E. Fertilization and Fruit Set.

Single fertilization was observed in a few ovules (Fig. 19). This has also been reported (7, 36, 37, 38) in ovules of other apple varieties. Examples of fertilization of only the egg cell and of fertilization of only the fusion nucleus were seen. Dorsey (11) has stated that single fertilization might be due to the formation of only one functional male gamete or to the absorption of the fusion nuclei as well as the antipodals. The writers suggest that the formation of only one functional male gamete is probably the more likely situation in the ovules of Delicious that were observed since male gametes were seldom found while the fusion nucleus appeared to be functional in practically all cases.

The enlarged fruits collected as "expected drops" four to seven weeks after pollination showed no signs of embryo abortion but contained many collapsed ovules which evidently had not been fertilized (Fig. 22, 23, 24). These fruits were expected to abscise in the second drop because of their low seed content and competition with other fruits on the cluster base. In Delicious the second drop is usually much lighter than the first (21, 31) except in those years when there is a heavy early set.

Howlett (21) has stated that the problem of fruit set in Delicious involves consideration of the factors responsible for the first drop which is severe in comparison to varieties as Jonathan and Grimes Golden. These factors are numerous, especially with reference to Delicious. Several environmental factors often contribute their share toward an unfavorable fruit set in this variety. Such factors as the lack of sufficient pollinizing insects, weather unfavorable for insect flight at bloom, inadequate soil minerals and nitrogen, freezing temperatures with resultant injury to the stigma, style and ovule itself (Fig. 25), and an unsatisfactory pollen supply may be cited. In certain years one of the environmental factors may be singled out as the main cause of unfavorable fruit set. Such an example would be the occurrence of a severe freeze in late spring with the killing of many flowers. However, more commonly the unsatisfactory fruit set is the result of the cumulative, complementary effect of several of these factors.

In addition to the above environmental factors, this work furnishes evidence that an inherent factor (or factors), peculiar to Delicious, is operative toward unsatisfactory fruit set with its cytological expression in delayed development and premature degeneration of the megagametophyte. The extent to which this inherent factor (or factors) affects fruit set is certainly of considerable importance since three of the ten (30 percent) ovules in each flower could be expected to contain immature or degenerating embryo sacs. Data have been obtained (6, 18) which indicate that the presence of 4 to 6 seeds is required per fruit in our apple varieties in order to set. If these seeds are eliminated at the onset due to immature and degenerating embryo sacs, and weather conditions are not too favorable for pollination and stigma receptivity is reduced, several more ovules may fail to be fertilized. This combination of unfavorable inherent and environmental factors would result in a low seed content below or near the critical number in this variety. On the other hand other diploid varieties which may have been subjected to the same weather conditions still set a satisfactory crop.

Although the seed content in these other varieties may likewise be reduced, the critical number has not been reached due to the absence of an unfavorable inherent factor (or factors) as in Delicious.

Furthermore, it might be surmised that with the Delicious variety a minimum of 6 or 7 seeds is required to set fruit. With 30 percent of the ovules shown to be "abnormal" to some extent, it is conceivable that the remaining ovules may be of a weaker constitution than ovules of other varieties, thereby requiring a larger number of fertilized ovules or seeds for fruit set. Further investigations need to be made in this regard.

As previously indicated the principal objective of this work was to obtain information concerning the precarious fruit setting behavior of Delicious and its mutations as compared with such dependable varieties as Golden Delicious and Rome Beauty. Ultimately, of course, some such basic inquiry is essential in order to determine whether or not the fruit grower can in fact take practical steps to overcome this serious shortcoming. These results, obtained by examination of the flowers at various stages up to and shortly after petal fall, not only provide a clue, but also give definite information concerning the extent of such irregularity. The frequency of such occurrence is extremely important and in this respect the data herein presented are unique in cytological studies with tree fruits. For it now becomes evident that these irregularities occurred with sufficient frequency to influence unfavorably the number of seeds developing in the fruit, a factor accountable in part for the unstable fruit setting. Thus these data help to explain the reason why Delicious and its mutations are less fruitful than varieties such as Jonathan, Golden Delicious, Gallia and Rome Beauty. Furthermore, it immediately becomes evident that when unfavorable environmental conditions are superimposed upon the original cytological irregularities, fruit setting is still further reduced. Under extreme conditions the variety may fail completely to develop a crop.

And so the question arises as to how the fruit grower is to live with these presumably inherited irregularities. These he cannot control. What then, from a practical angle, can he do to ameliorate those conditions which he can alter? In other words how can he compensate by control of environmental factors for those other factors about which he can do little, to prevent frost injury (once the planting is established), to reduce rainfall during bloom or maintain temperatures favorable to bee flight during bloom and for fruit development later. Yet he can make certain adjustments which will to some extent minimize the severity of these restrictive influences. And in view of the situation these adjustments become even more imperative.

Thus all the old general provisions for increasing fruit are in the case of Delicious and its mutations even more essential. Furthermore, they must be more precisely, more specifically carried out. To begin with there are the provisions for establishing a new planting. Only the most frost-free sites should be planted to these varieties. Then too the most favorable position of the site in terms of elevation and air drainage should be given over to the exacting requirements of Delicious. Next the planting plan should provide for a much higher proportion of pollinizing trees than has formerly been recommended. It is to be remembered that Delicious and its mutations are completely self-unfruitful and therefore Delicious should be planted immediately adjacent to its pollinizer. Thus two rows of Delicious may be planted together providing that a pollinizer is planted at either side.

Then the pollinizers themselves are to be scrutinized. Their blooming season must coincide with that of Delicious. Their pollen must be viable and the two varieties compatible. Suggested pollinizers are Jonathan, Golden Delicious, McIntosh, Cortland and Franklin. Varieties considered unacceptable, due to the fact that blooming seasons do not coincide to a sufficient extent, are Rome Beauty, Gallia Beauty Red Rome, Ruby or any Rome Beauty mutation. Turley, Stayman Winesap and its mutations such as Blaxtayman, Staymared, Scarlet Staymared are also excluded because their pollen is not viable and Melrose because it is incompatible with Delicious. The work herein reported concerning the relatively short period of time during which a Delicious flower is receptive to pollination serves to emphasize the care with which the pollinizing varieties must be selected.

In an established planting of Delicious the possible adjustments are naturally more restricted than with a new planting. Topworking now becomes the best means of bringing pollinizers into closer proximity. There is no reason why topworking trees 10 to 20 years old cannot be satisfactorily carried out with resulting benefits in improved fruit setting.

But the most immediately possible means of improving the Delicious situation lies in strengthening the provisions for pollinizing insects. The requirements of the heavier setting varieties can by no means be considered as a yardstick in this case. The introduction of more and stronger colonies must compensate for the lack of activity in cool and unfavorable weather. Wherever there is any question of doubtful fruit set one strong colony should be maintained per acre.

From the nutritional angle it must be admitted that the data reported in this publication offer less help in improving the situation than had been hoped. Contrary to expectation there seemed to be little

correlation between the degree of vigor of the tree and the incidence of those cytological disturbances which affect fruit set. Actually there was more difference between the flowers in a single cluster as to the incidence of this irregularity than there was between one tree and another.

But although these irregularities occurred without apparent reference to the vigor of the tree the fact remains that under nitrogen deficient conditions fewer flowers at full bloom have the capacity for fertilization. And it is commonly accepted that a shortage of nitrogen and other elements essential for plant growth will reduce fruit set. Thus again it becomes necessary to use the nutritional factor as a means of compensating for the irregularity which itself remains unaffected by nutrition. And so proper nutrition which is important for fruit setting generally becomes a "must" in the case of Delicious and its mutations.

Regular pruning should also be useful in so far as it prevents the excessive "snowball bloom" and the consequent almost complete abscission of flowers. In addition improved water supply resulting from regular annual pruning is also indicated in this case. In fact any practice which conserves water, such as frequent cutting of the grass, breaking up of the sod, use of mulch or irrigation will help to maintain the more uniform growth which results in more uniform production. In other words if any variety is to be neglected, overlooked until another season, Delicious is not the one to fall into this category.

But in spite of all these hazards satisfactory crops of the Delicious apple have been obtained by some Ohio growers, and it is reasonable to suppose that other growers who have previously been disappointed may, in the future, by following the suggestions outlined above be able to produce satisfactory crops of Delicious. For it is probably safe to say that the Delicious apple is still second to none so far as consumer preference is concerned. Realizing this fact and recognizing the inherent handicaps of the variety the grower must plan the necessary adjustments and compensations in order to insure the profitable commercial production of Delicious.

SUMMARY

1. Flowers and young fruits of the Delicious apple variety were collected at anthesis and 24-hour intervals thereafter from trees located in four different orchards about the State of Ohio and cytological examination was made of the embryo sacs and embryos contained within.

2. Samples were obtained of flowers in the various positions on the cluster base, from weak and strong spurs, and from individual trees.

3. In addition to a study of the various types of abnormalities observed, the frequency of such abnormalities was recorded on a percentage basis.

4. A considerable percentage of the ovules from flowers collected at anthesis contained embryo sacs which were delayed in development or showed signs of premature degeneration.

5. Flowers collected at anthesis from the different positions on the cluster base contained an increasing percentage of ovules with embryo sacs which were not fully differentiated at anthesis in the following order—terminals, laterals in the axil of a leaf, laterals with no subtending leaf or bract, and smallest laterals.

6. Flowers collected at anthesis from strong and weak spurs contained ovules with embryo sacs which showed little difference in the percentage of irregularities in the development of the sacs when flowers from all of the cluster base positions were taken collectively. Apparently the development of the embryo sac is influenced to a greater extent by flower position than by spur vigor.

7. The difference in percentage of fully differentiated eight-nucleate embryo sacs from flowers collected at anthesis from individual trees was relatively small when flowers from all of the cluster base positions were taken collectively. In all of the trees from which collections were obtained there was considerable immaturity and early degeneration of the embryo sacs.

8. It is suggested that delayed development and early degeneration of the embryo sac nuclei at and subsequent to anthesis has a genetic basis in this variety.

9. The percentage of ovules containing a degenerating embryo sac increased considerably in non-pollinated flowers 72 hours after anthesis.

10. The occurrence of fertilization was observed to be greatly reduced in ovules of flowers in which pollination had been delayed 48 hours after anthesis. This is attributed largely to the early loss of receptivity of the stigmas.

11. Fertilization was seldom observed in embryo sacs in less than 72 hours after pollination. This observation, in addition to early degeneration of the embryo sac, is believed to account to a considerable extent for the erratic fruit setting of Delicious.

12. The percentage of ovules with two or more embryo sacs increased daily after anthesis. The origin of multiple embryo sacs was observed to occur in two ways—from a common or from different megaspore mother cells.

13. Single fertilization of the embryo sac was observed. It is suggested that this is probably due to the formation of only one functional male gamete.

14. It appeared that the abscission of young fruits which were expected to fall during the "June drop" was not due to embryo abortion, but rather to an inadequate number of ovules having been fertilized and to competition with the fruits expected to remain on the tree.

15. With this discovery that genetic factors exist which so unfavorably affect the fruit set in the Delicious apple, it becomes imperative that the grower of this variety must be cognizant of the increased need of regulating those environmental factors within his power to help to insure a commercial fruit set. Important among these is to provide for the most satisfactory conditions for pollination.

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Fig. 2.—Longitudinal section through an ovule (19-A-6-11) of a flower collected at anthesis containing a conspicuous megaspore mother cell. (1425 X)

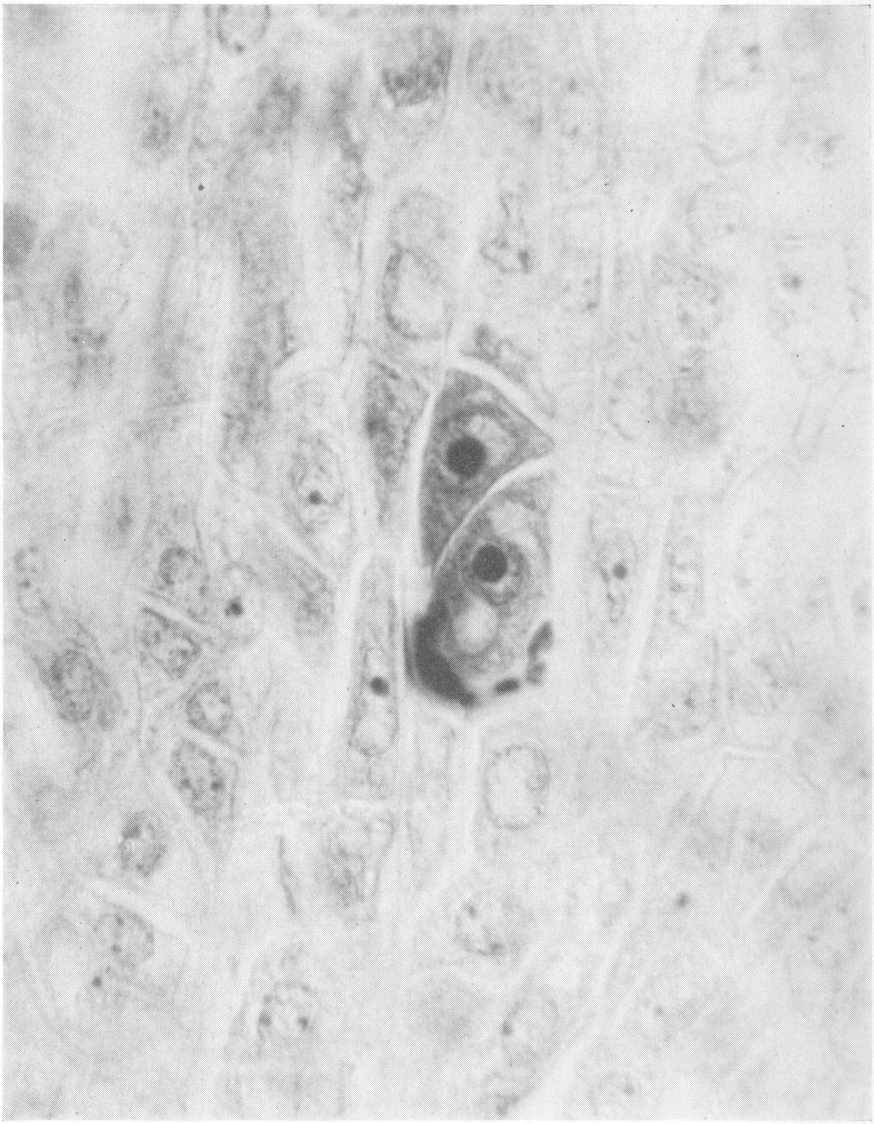

Fig. 3.—Longitudinal section through an ovule (33-A-1-1) of a flower collected at anthesis containing megaspores. The fourth megaspore was in another plane and found in the adjoining section. (1425 X)

Fig. 4.—Longitudinal section of an ovule (41A-A-4-8) of a flower collected at anthesis containing a degenerating chalazal megaspore. (1425 X)

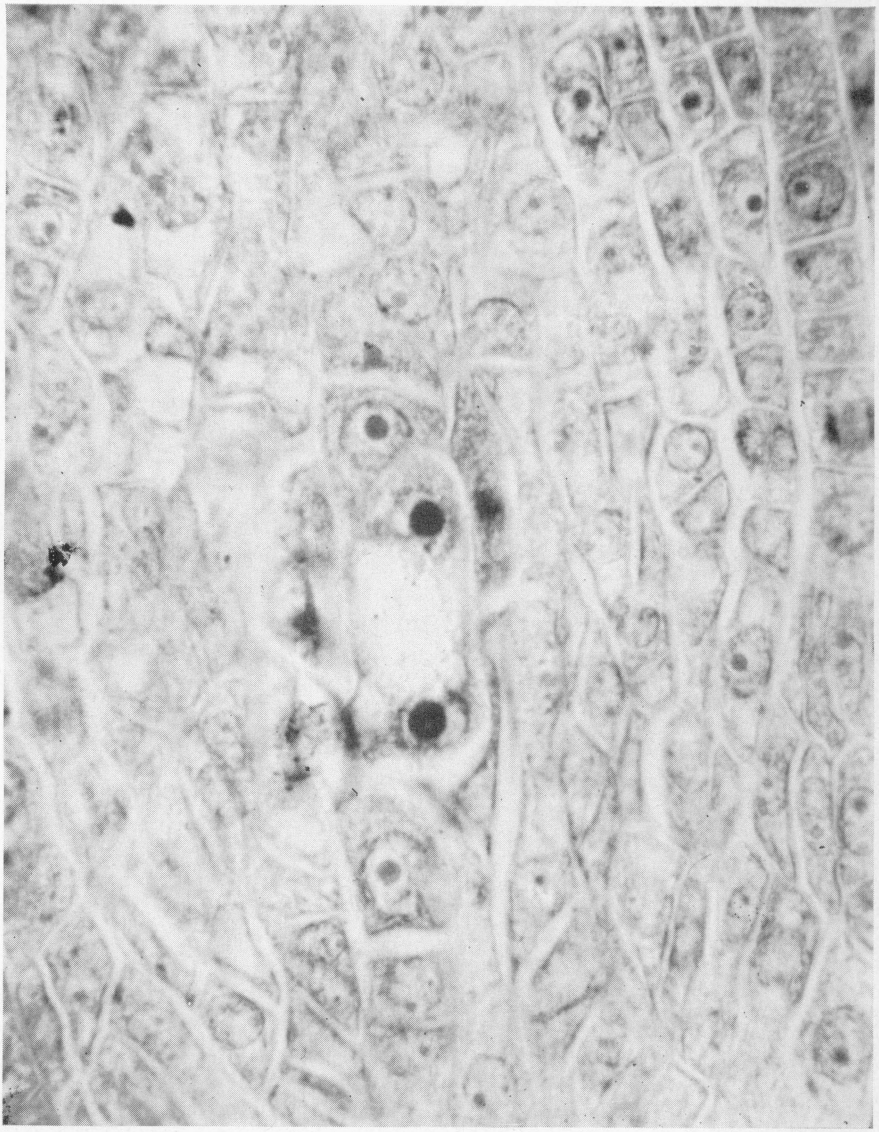

Fig. 5.—Longitudinal section of an ovule (42-A-5-10) of a flower collected at anthesis containing a two-nucleate embryo sac. (1100 X)

Fig. 6.—Longitudinal section through an ovule (48-A-7-4) of a flower collected 48 hours after anthesis containing a four-nucleate embryo sac. Two of the four nuclei were in an adjoining section. (1425 X)

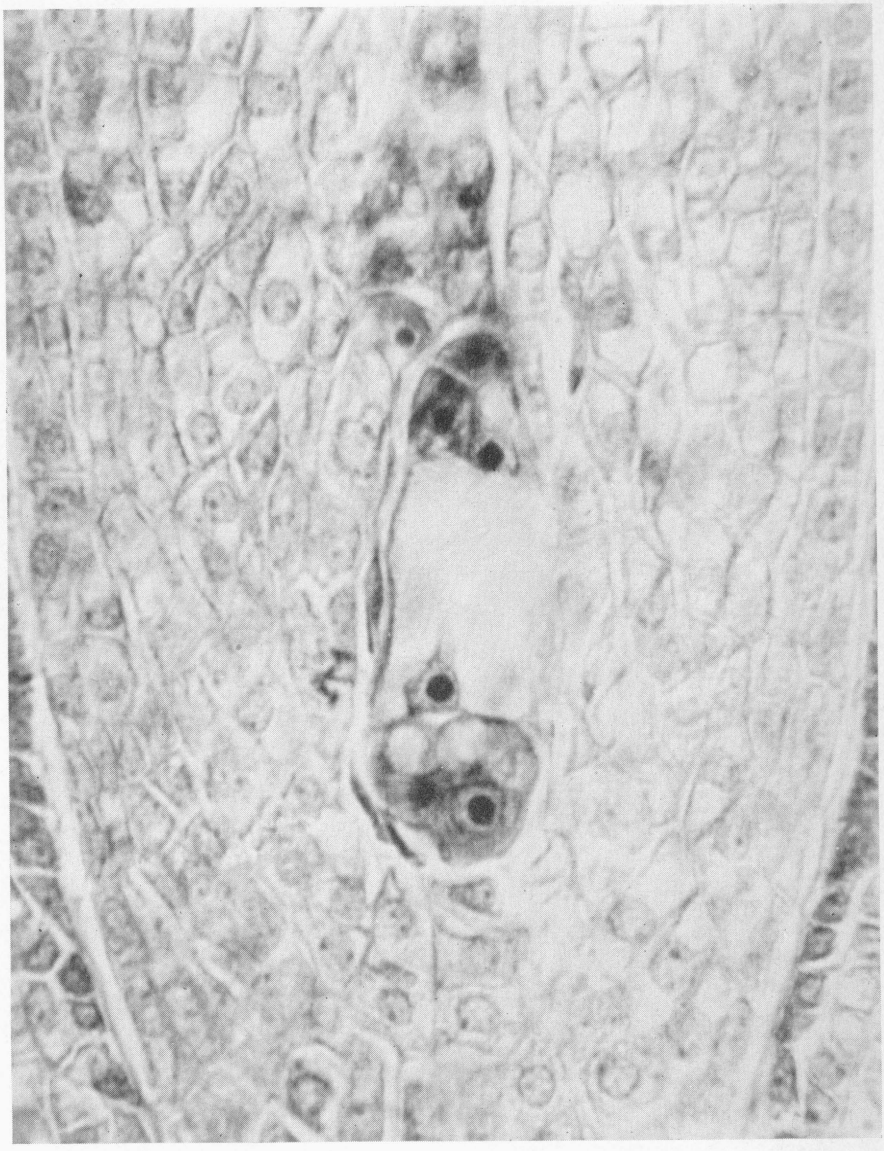

Fig. 7.—Longitudinal section through an ovule (40A-B-3-6) of a flower collected at anthesis containing an eight-nucleate embryo sac which has not fully differentiated. (1425 X)

Fig. 8.—Longitudinal section through an ovule (37B-A-2-3) of a flower collected at anthesis containing a fully differentiated eight-nucleate embryo sac. The three antipodal cells are present in an adjacent section. (1300 X)

Fig. 9.—Longitudinal section through an ovule (53-A-2-5) from a non-pollinated flower collected four days after anthesis. The polar nuclei have fused. (1166 X)

Fig. 10.—Longitudinal section through an ovule (26-A-3-5) of a flower collected at anthesis containing an embryo sac in which the egg was degenerating. (1425 X)

Fig. 11.—Longitudinal section through an ovule (21-A-3-4) of a flower collected at anthesis containing an embryo sac in which the entire contents have degenerated. (1425 X)

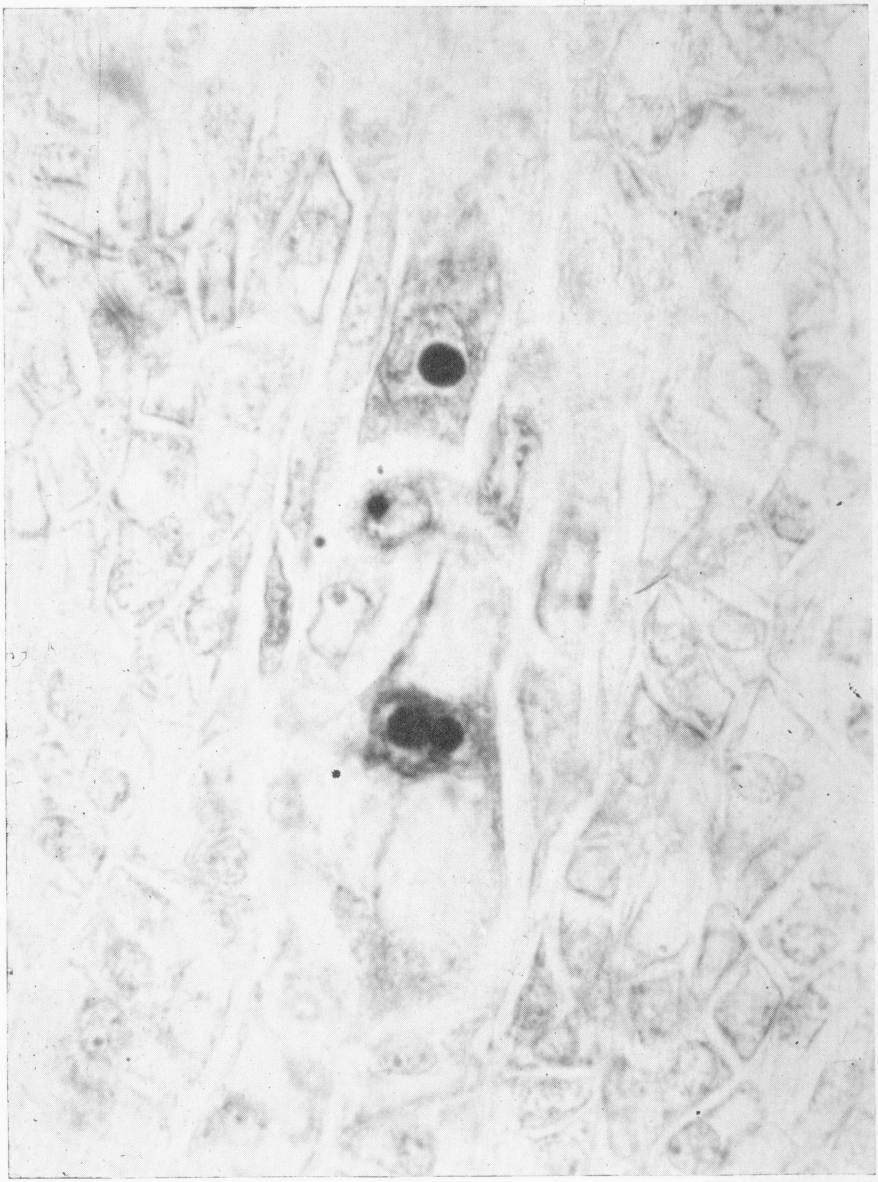

Fig. 12.—Longitudinal section through an ovule (3-A-3-6) of a flower collected at anthesis containing a megaspore mother cell above an eight-nucleate embryo sac. Only the polar nuclei of the embryo sac are seen in this section. (1166 X)

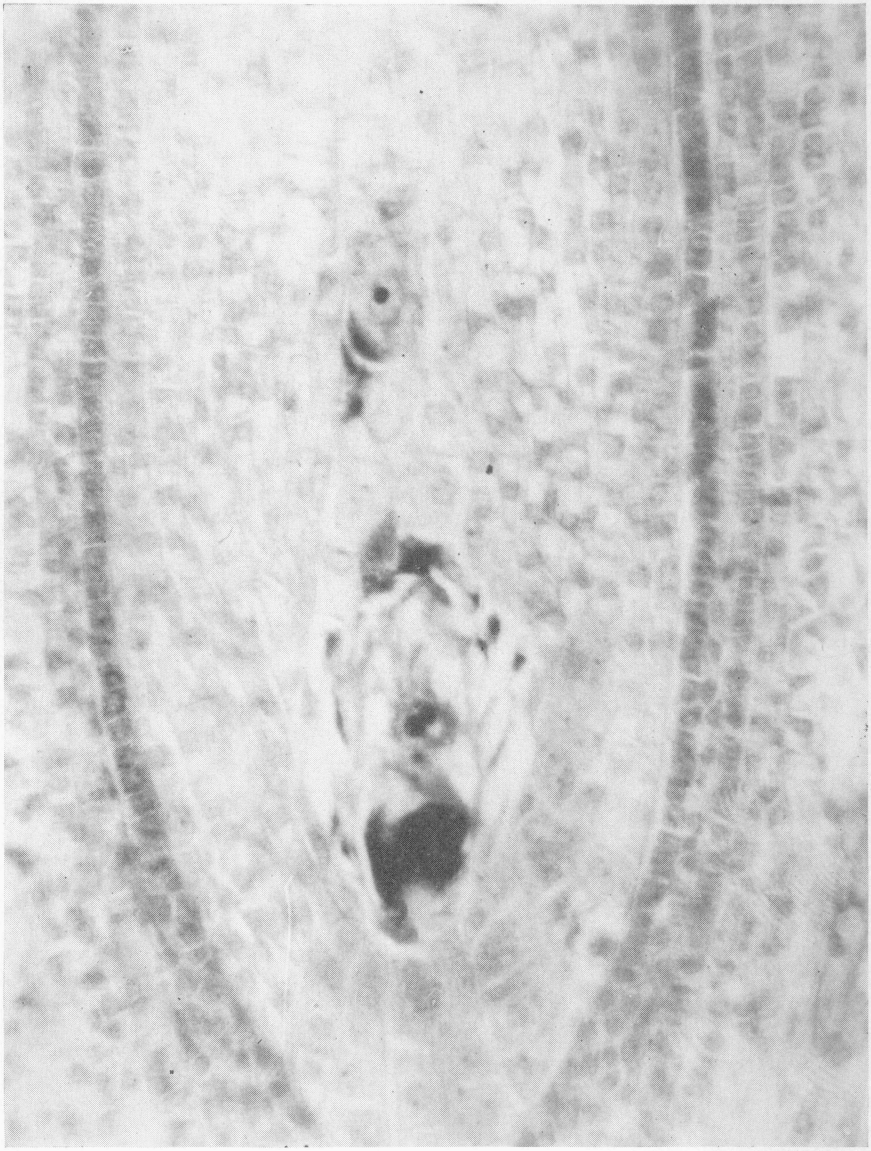

Fig. 13.—Longitudinal section through an ovule (304-A-2-3) of a flower pollinated 24 hours after anthesis and collected two days after pollination. The ovule contains megaspores above an eight-nucleate sac. (552 X)

Fig. 14.—Longitudinal section through an ovule (12-A-2-2) of a flower collected at anthesis containing megaspores above an eight-nucleate embryo sac. The egg is seen below the fused polar nuclei while the synergids and three other megaspores are in an adjoining section. (1300 X)

Fig. 15.—Longitudinal section through an ovule (8-A-3-2) of a flower collected at anthesis containing two eight-nucleate embryo sacs situated side by side. Three nuclei, the polars and a synergid, are shown in the sac on the left, and four nuclei, the polars, egg and one synergid, in the sac on the right. (1166 X)

Fig. 16.—Longitudinal section through an ovule (312-A-1-1) of a flower pollinated three days after anthesis and collected two days after pollination. The ovule contains a large and a small embryo sac. (633 X)

Fig. 17.—Longitudinal section through an ovule (105-A-3-4) of a flower collected at anthesis containing three embryo sacs. (647 X)

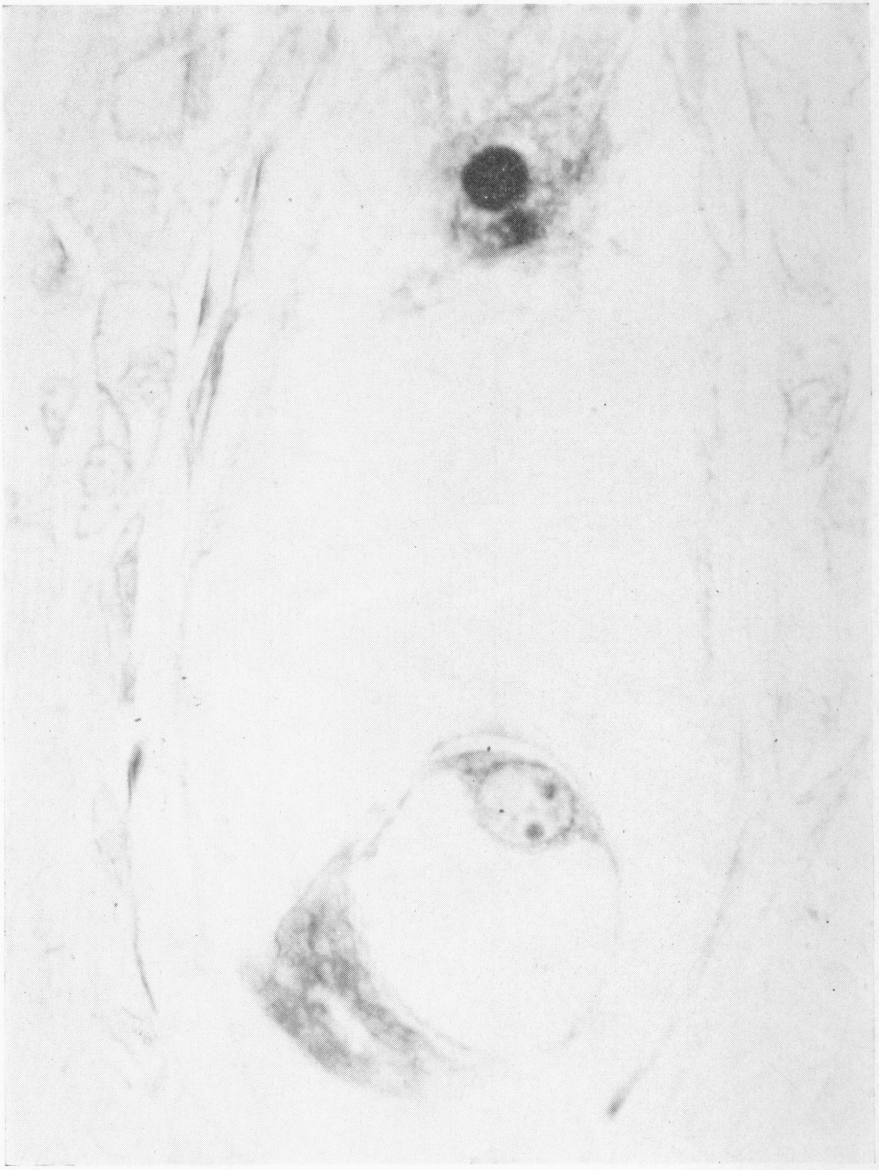

Fig. 18.—Longitudinal section through an ovule (320-A-3-6) of a flower pollinated 48 hours after anthesis and collected two days after pollination having indications of double fertilization. (1425 X)

Fig. 19.—Longitudinal section through an ovule (66-A-6-9) of a flower pollinated four days after anthesis and collected three days after pollination in which division of the primary endosperm nucleus was occurring. The egg appeared unfertilized. (544 X)

Fig. 20.—Longitudinal section through a developing seed (325A-A-5) of a young fruit which originated from a flower that was pollinated two days after anthesis and collected three days after pollination having indications of double fertilization. (635 X)

Fig. 21.—Longitudinal section through a seed (331-A-3-6) of a young fruit which developed from a flower pollinated two days after anthesis and collected five days after pollination containing a several-celled embryo. (1425 X)

Fig. 22.—Longitudinal section of a young fruit which developed from an open-pollinated flower (79A-A-1) collected six weeks after anthesis, containing four collapsed ovules. (39 X)

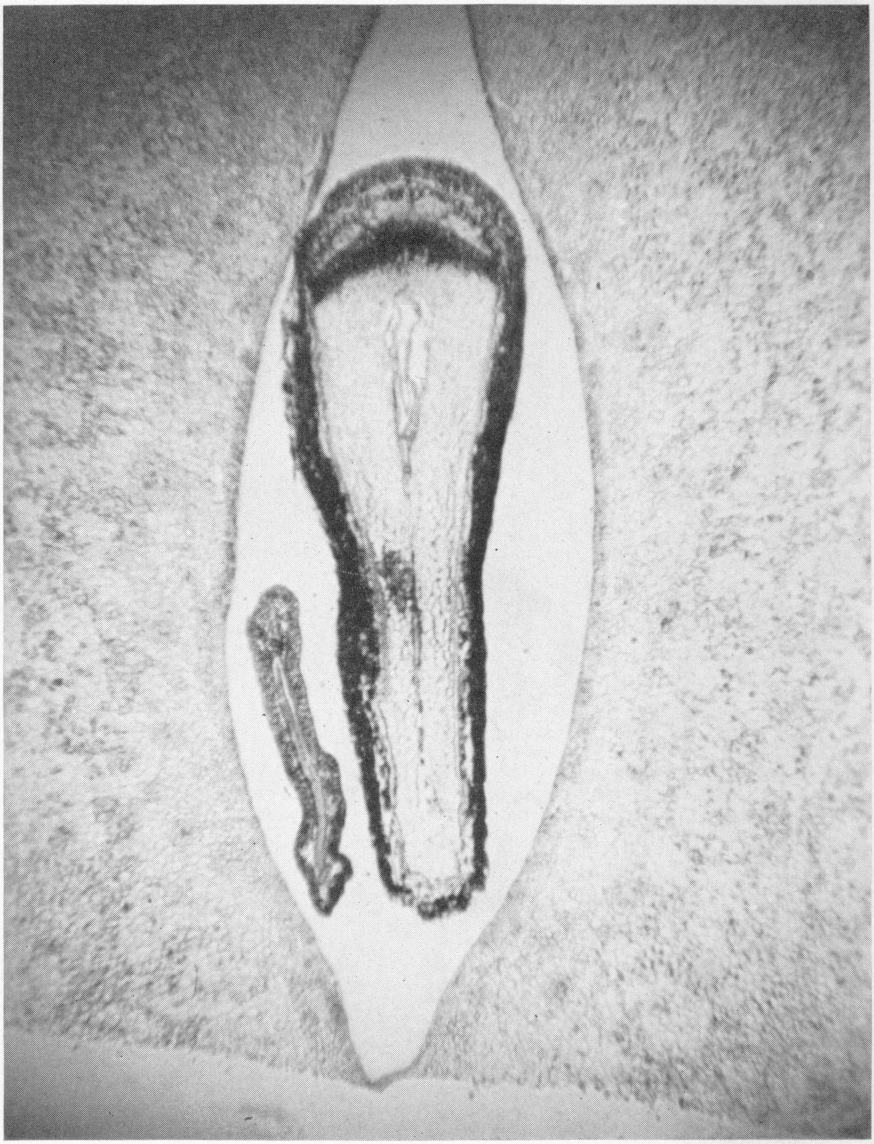

Fig. 23.—Longitudinal section of a young fruit from an open-pollinated flower (79-A-2) collected six weeks after anthesis containing a young seed and a collapsed ovule. (39 X)

Fig. 24.—Longitudinal section through a young fruit from an open-pollinated flower (78A-A-15) collected six weeks after anthesis containing a young seed showing embryo and endosperm nuclei. (300 X)

Fig. 25.—Longitudinal section through a flower (22A-A-5) collected at anthesis showing two ovules. The one on the right contains considerably more frost damage than the one on the left. (196 X)